Supplementary methods:

Construction of fluorescent tagged proteins

GFP fusion proteins were constructed by cloning in vector pCN19 as described earlier [1]. Briefly, the ORF along with ~300 bp 3' UTR was amplified from the genome of *C. neoformans* strain KN99 and cloned in the vector pCN19 using either *Bam*HI alone or *Bam*HI and *Spe*I. This lead to expression of N-terminally GFP tagged protein under the constitutive histone H3 promoter. Resulting plasmids were then used to transform KN99 strain of *C. neoformans* by the biolistics method as described previously [2] and selected on YPD medium containing 100 µg/ml of nourseothricin (NAT). The transformants were screened by fluorescent microscopy. To tag Nuf2 with GFP at the C-terminus at its native locus (strains SHR515, SHR516), 1 kb region of the gene up to the penultimate codon was cloned using *Xba*I and *Pst*I in a vector with GFP and NAT. Approximately 1 kb of the 3'UTR region was cloned behind NAT using *Sal*I and *Apa*I . The cassette was released from plasmid using *Xba*I-*Apa*I and transformed into KN99.

To tag Cse4 with mCherry at the N-terminus in the strain LK275, a fragment of *CSE4* ORF followed by 477 bp with flanking cleavage sites for *Nhe*I and *Pac*I was PCR-amplified and ligated into pLKB49 (which contains the neomycin resistance gene and is used to express mCherry-tagged chimeras from a constitutive GPD1 promoter) and resulted in plasmid pLKB70. A similar construct was also made that resulted in mCherry-Cse4 expression from the endogenous *CSE4* promoter (pLKB74) by replacing GPD1 promoter in pLKB49 with a fragment of 530 bp upstream of *CSE4* ORF and containing flanking XhoI and FseI cleavage sites. This construct (pLKB74) was utilized to generate strains CNV101, CNV111, CNV114, LK317, and LK353.

To tag proteins with mCherry at the C-terminus in CNV102 (and derived strains), CNV103 (and derived strains), and CNV119, the overlap PCR strategy was used as described previously [1]. Approximately, 1 kb gene sequence up to the penultimate codon and 1 kb sequence downstream of the stop codon was amplified from the KN99 genome. A 3.3 kb long sequence fragment containing mCherry along with neomycin gene was amplified from a plasmid, pLKB25 [1]. Three parts were purified separately and used as template for overlap PCR. The overlap product of 5.3 kb was used to transform H99α or KN99**a** strains and transformants were selected on YPD medium containing 200 μg/ml of G-418. The transformants were screened by fluorescence microscopy and PCR to show the integration of mCherry-encoding sequence at 3' end of the target gene.

3D image analysis

Z-section analysis and three-dimensional (3D) reconstruction of images obtained with the DeltaVision system were performed using Imaris software (Bitplane AG, CT). To model the Cse4-Tubulin doublet at metaphase an object reconstruction with "surfaces" function was used. To estimate the positions and the number of centromeres, spot detection algorithm was performed. The mCherry-Cse4 signals were modelled as spheres. The threshold for the signal intensity was set manually to exclude background fluorescence. Mean fluorescence signals corresponding to each mCherry-Cse4 spot for a given cell were summed up and divided by the theoretical number of chromosomes (14 for the haploid and 28 for the diploid) resulting in an approximate signal corresponding to a single centromere. This allowed for an estimation of the number of centromeres in each cluster as indicated in Figure S1A. This estimation was based on 2 assumptions: 1) fluorescently-tagged Cse4 has incorporated only at centromeric regions and 2) the number of molecules of Cse4 in each centromere is constant. Our fluorescent Cse4 constructs

were expressed ectopically from a constitutive promoter (tagging of an endogenous *CSE4* gene was not possible for technical reasons). While CENP-A marks centromere chromatin in eukaryotes, it is theoretically possible that an excess of mCherry-Cse4 has associated with non-centromeric regions. However, co-localization of GFP-Cse4 and Mif2-mCherry (a fluorescent chimera of an endogenous *MIF2* gene) shows a complete overlap with no Cse4-exclusive regions. Moreover, an excess of CENP-A has been shown recently to be effectively excluded from non-centromeric regions [3-4]. Thus, a significant non-centromere association of mCherry-Cse4 is unlikely.

Budding index calculation

Budding index was calculated for 100 cells with respect to each kinetochore layer protein. The cell diameter was measured for the mother and bud from the neck region using either the Image Pro-plus software or LSM software. The diameter of bud was divided by mother cell diameter and ratio, budding index, was obtained.

Electron microscopy

The electron microscopy samples were prepared as described previously [5]. Briefly, *C. neoformans* cells collected by centrifugation were sandwiched between two copper disks; snap frozen by melting propane cooled with liquid nitrogen, and freeze-substituted in acetone containing 2% osmium tetroxide at -80°C for 2–3 days. They were embedded in epoxy resin, and ultrathin sections were examined in a JEM 1200EX transmission electron microscope (JEOL, Tokyo).

Nocodazole treatment

The cells were grown overnight in YPD and synchronized to get unbudded cells as described previously [6]. The culture was divided in two halves, half of which was treated with DMSO solution. The other half was treated with DMSO + Noc at final concentration of 1 µg/ml. Initially cultures were treated for 1 hour under low oxygen conditions to maintain unbudded state. Next cultures were diluted into fresh medium and grown with aeration for 70 minutes or 3.5 hours either in the presence of Noc or DMSO. The cells were collected and placed on 2% agarose patch (supplemented with either Noc or DMSO for the control cells) on the microscope slide and imaging was performed.

Supplementary references

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